Receptor orphans find a family

A family of ligands has been identified for the largest group of receptor protein-tyrosine kinases — the hitherto 'orphan' EPH receptor subfamily — and the functions of these receptors and ligands are starting to be elucidated.

A characteristic feature of multicellular organisms is that cells communicate with each other to coordinate their activities. This communication is mediated by cell-surface receptors and their ligands. The known receptors fall into several distinct types, each with a characteristic mechanism of transducing an extracellular signal into an intracellular one. One of these major receptor types is the receptor protein-tyrosine kinase type — these receptors have intracellular kinase domains that are activated in response to ligand stimulation, leading to autophosphorylation of tyrosine residues; the phosphorylated tyrosines in turn bind to, and activate, a number of signalling molecules, turning on multiple signalling pathways inside the cell.

The various receptor protein-tyrosine kinases have been divided into subfamilies on the basis of their sequences. The most recent classification [1] delineated 14 distinct subfamilies of receptor protein-tyrosine kinases, including the epidermal growth factor (EGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF) and insulin receptors. The largest group is the 'EPH' subfamily, which until recently were ligand-less, 'orphan' receptors. Ligands for EPH-like receptors have, however, now been identified, and constitute a rapidly expanding family of glycosylphosphatidylinositol (GPI)-linked or transmembrane molecules. Elucidation of the functions of these receptor–ligand systems is just beginning, but it seems likely that they play a number of important roles, particularly in the development of the nervous system.

The EPH receptor subfamily

EPH subfamily receptors are characterized by an extracellular region that includes an immunoglobulin-like domain, a cysteine-rich sequence that is not related to those in EGF or insulin receptors, and two fibronectin type III repeats. There are 20 cysteines in the extracellular region that are conserved among all the subfamily members. The cytoplasmic region contains a single tyrosine kinase domain and a non-catalytic tail of about 100 amino acids [1]. The prototypical member of this family, EPH, was identified in a human genomic DNA library during a search for gene sequences homologous to the tyrosine kinase domain of the viral oncogene v-*fps* [2]. Subsequently, several members of this family have been identified from various vertebrate species, including humans, mouse, rat, *Xenopus* [3] and zebrafish [4]. To date, 13 distinct members of this family have been

Human ligand	Human gene symbol	Mouse ligand	Mouse gene symbol
B61, LERK-1, EFL-1	*EPLG1*		*Epl1*
Elk-L, LERK-2, EFL-3	*EPLG2*	Cek5 L	*Epl2*
LERK-3, Ehk1-L, EFL-2	*EPLG3*		*Epl3*
LERK-4	*EPLG4*		*Epl4*
huHtk L, LERK-5	*EPLG5*	muHtk L	*Epl5*
	EPLG6	ELF-1, Cek7 L	*Epl6*
AL-1	*EPLG7*		*Epl7*

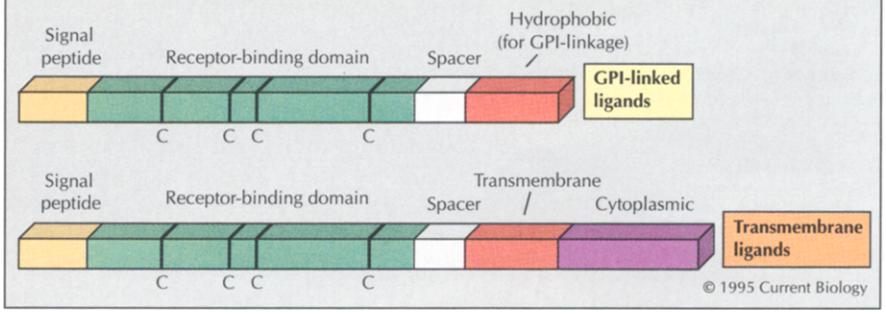

Fig. 1. Ligands for EPH subfamily receptors. The ligand gene names were kindly provided by Doug Cerretti, and have been approved by the mouse and human gene nomenclature committees. The topologies of the two types of ligand — GPI-linked (yellow shading) and transmembrane (orange shading) — are shown below the table listing the known ligands.

discovered, comprising nearly a quarter of all known receptor protein-tyrosine kinases.

Expression of EPH subfamily receptors

Initial clues to the likely functions of proteins discovered on the basis of their gene sequences can come from knowledge of where they are expressed. Several members of the EPH subfamily are predominantly expressed in the adult brain. These include Elk, Eek, Cek4, Cek5, Sek, Ehk-1 and Ehk-2. Other members, however, such as EPH, ECK, HTK, Cek9 and Cek10, have a broader tissue distribution. The tissue distributions of many of the EPH-like receptors have recently been reviewed by Tuzi and Gullick [5]; three EPH-like receptors not described in that review are Mdk1/Ehk-3/HEK11 [6–8], Ehk-2 [9] and HTK/Myk-1 [10,11].

Mdk1/Ehk-3/HEK11 is expressed widely in the embryonic brain as well as in a variety of other tissues, including kidney, lung, heart and liver. In the adult, the expression is diminished and more restricted to the brain, testes and spleen [6–8]. Ehk-2 is almost exclusively expressed in the brain, both in the embryo and in the adult [9]. HTK is widely expressed in the embryo as well as the adult; it is found in fetal heart, lung, liver, kidney and brain. In the adult, HTK is expressed in placenta, kidney, liver, lung, skeletal muscle, heart and pancreas, with no detectable expression in brain. HTK mRNA is also detected in primitive hematopoietic cells and in cell lines derived from myeloid cells, but not in those derived from lymphoid cells [10].

Ligands for EPH subfamily receptors

The first ligand to be identified for an EPH-like receptor was B61, which was found to bind and activate ECK [12,13]. Since then, several other ligands related to B61 have been discovered by expression cloning, using the extracellular domains of EPH-like receptors expressed as immunoglobulin or alkaline phosphatase fusion proteins to detect ligand expression. Including B61, seven ligands in this family have been identified to date (Fig. 1). Interestingly, five of the ligands appear to be GPI-linked, whereas the other two have transmembrane domains.

An alignment of the amino-acid sequences of the known ligands for EPH-like receptors is shown in Figure 2. As suggested by Kozlosky *et al.* [14], each ligand molecule can be divided into four regions: a signal peptide, a receptor-binding region, a spacer region and a hydrophobic region (Fig. 1). The only region of significant identity over the whole family is the receptor-binding region, in which there are four cysteines that are conserved among all known ligands for EPH subfamily receptors. A site for *N*-linked glycosylation, located near the amino terminus of the mature peptide, is also highly conserved, being present in all EPH-subfamily receptor ligands except LERK-2/Elk-L/EFL-3. Htk L/LERK-5 and AL-1/RAGS, and LERK-3/Ehk1-L/EFL-2 and ELF-1/Cek7 L, contain one and two additional putative *N*-linked glycosylation sites, respectively. The two transmembrane ligands also show extensive sequence identity in their spacer, hydrophobic transmembrane and cytoplasmic regions.

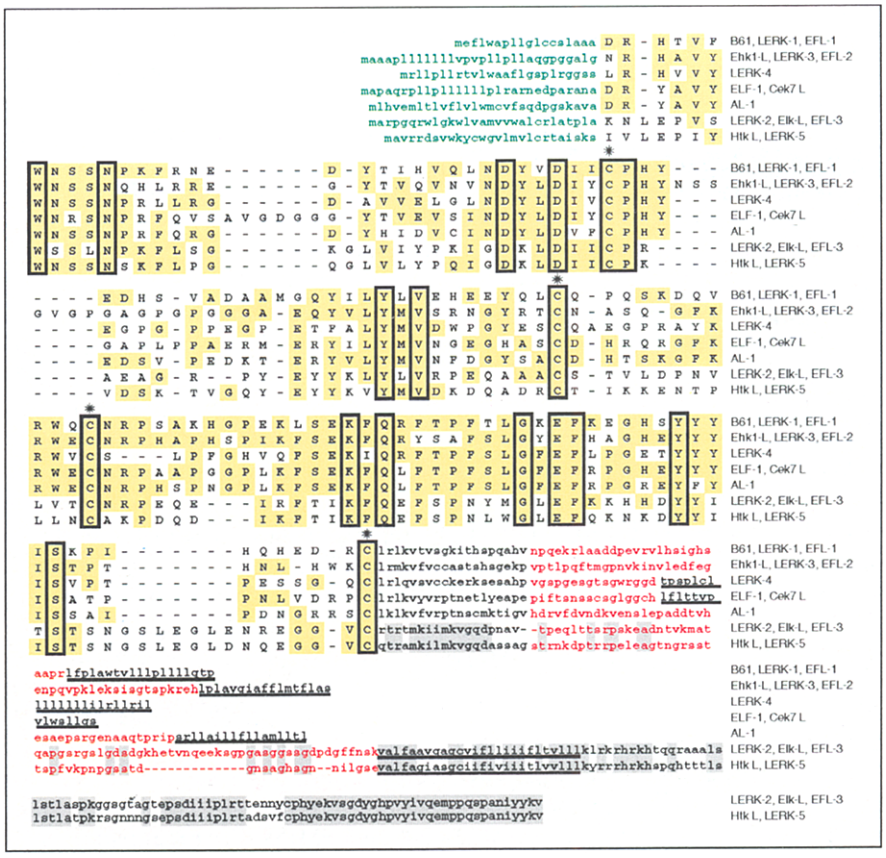

Fig. 2. Alignment of the known sequences of B61-related ligands of EPH subfamily receptors. All sequences shown are human, except ELF-1/Cek7 L, for which only the mouse sequence has been published. The receptor-binding domains of the GPI-linked ligands, and the entire sequences of the transmembrane ligands, have been aligned, using the MegAlign progam and visual inspection. The signal peptides are shown in green, the receptor-binding domains in black with regions of sequence similarity highlighted in yellow, and the spacer regions are red. The hydrophobic and transmembrane regions are underlined; residues conserved among all ligands are boxed; and conserved cysteines are indicated with a star. The spacer, transmembrane and cytoplasmic regions of the two transmembrane ligands show significant sequence identity to each other and are shaded grey.

Table 1. Expression patterns of the identified ligands of EPH subfamily receptors.

Ligand	Species examined	Expression pattern
B61/LERK-1/EFL-1	Human	Expressed in umbilical vein endothelial cells, keratinocytes and fibroblasts.
	Mouse	In the embryo: high levels in lung and gut; scattered expression in facial bones, salivary glands and CNS; low levels in the thymus. In the adult: high levels in lung; low levels in the thymus.
	Rat	Expressed primarily in non-neuronal tissues in adults, including lung, spleen, liver, muscle, heart and skin.
LERK-2/Elk-L/EFL-3	Human	Expressed in heart, placenta, lung, liver, skeletal muscle, kidney and pancreas. Not detectable in brain.
	Rat	Widely expressed in neuronal and most non-neuronal tissues, including heart, lung, liver, muscle, kidney, spleen, thymus, skin and ovary.
LERK-3/Ehk1-L/EFL-2	Human	Expressed in fetal heart, brain, lung, liver and kidney. In the adult, expressed in brain, skeletal muscle, spleen, thymus, prostate, testis, ovary, small intestine, colon and peripheral blood leukocytes.
	Rat	Almost exclusively expressed in the CNS and the skin.
LERK-4	Human	Expressed in fetal heart, lung, and kidney. In the adult, expressed in spleen, prostate, ovary, small intestine and colon.
Htk L/LERK-5	Human	Widely expressed both in fetal and adult tissues. Expressed in fetal brain, lung, kidney, and liver. In the adult, it is expressed in almost all organs and tissues. Also expressed in a fetal liver stromal cell line that is capable of producing multilineage expansion of hematopoietic stem cells.
ELF-1/CEK7 L	Mouse	Expressed in embryonic midbrain, hindbrain, first and second branchial arches, somites and limb buds.
AL-1/RAGS	Human	Expressed in the adult brain, heart, placenta, lung and kidney. Predominantly expressed in astrocytes rather than neurons.

The references for the above data are: B61/LERK-1/EFL-1 [13,15,16,25]; LERK-2/Elk-L/EFL-3 [15,16,26]; LERK-3/Ehk1-L/EFL-2 [14,16]; LERK-4 [14]; Htk L/LERK-5 [27,28]; ELF-1/Cek7 L [29,30]; AL-1/RAGS [23,24].

Expression of EPH-like receptor ligands

The mRNA expression patterns of the EPH-like receptor ligands are summarized in Table 1. Most of the data come from northern-blot analyses of whole tissues. It is apparent that, although many of the receptors are restricted in expression to the brain in adults, the ligands seem to have a more widespread expression. As the expression patterns of the ligands are generalized and not restricted to any preferred tissues, no obvious inference can be drawn about their likely roles *in vivo*. None of the ligand expression patterns exactly matches that of any EPH-like receptor.

It is known, however, that the ligand B61 is an immediate–early response gene in endothelial cells — its expression is induced by proinflammatory factors such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) and lipopolysaccharide (LPS) [13]. The ligand LERK-2 has similarly been shown to be induced by TNF-α in endothelial cells [15]. Whether other ligands are also induced by such proinflammatory factors has not yet been determined.

Biological functions

It is clear from studies in which receptor-binding affinities have been measured that any particular ligand in this family has affinity for many (or all) of the EPH-like receptors. Receptor activation, as measured by autophosphorylation, varies depending upon how the assay is performed. Experiments with artificially clustered, membrane-bound or soluble ligands give differing results, with the former two assay methods tending to show greater activity and promiscuity ([16] and R.A.L., unpublished observations). Which *in vitro* assay is the most relevant to function *in vivo* has yet to be determined. One would assume that both soluble and membrane-bound ligands have physiological functions. The determination of which receptor–ligand pairs are relevant *in vivo* will therefore depend upon other data, such as colocalization or genetic analyses.

The *EPH* gene has been shown to have transforming potential when overexpressed in NIH3T3 cells [17]. It has been found to be overexpressed, but not amplified, in a few tumor cell lines [17]. ERK was similarly found to be overexpressed in various tumors of epithelial origin, especially gastric carcinomas [18]. This suggests that overexpression of some of the EPH-like receptors may be involved in oncogenic transformation, as has been seen in the case of many other receptor protein-tyrosine kinases. The activation of a chimeric molecule consisting of the EGF extracellular domain and the Elk transmembrane and cytoplasmic domains, however, did not lead to proliferation of NIH3T3 cells [19]. At present, the data

indicate that EPH subfamily receptors do not activate proliferative pathways in cultured fibroblasts or endothelial cells [19,20]. This may be one of the reasons why identification of signalling pathways in the case of EPH subfamily receptors has been slow compared to other, mitogenic, receptor protein-tyrosine kinases.

As some of the ligands are expressed only when endothelial cells are activated by proinflammatory factors, such as TNF-α, IL-1 and LPS, it was expected that the activation of EPH subfamily receptors would be involved in certain aspects of inflammation. In this regard, B61 has been shown to induce an angiogenic response *in vivo* [20]. B61 also induces the migration of endothelial cells [20], which may be mediated by phosphatidylinositol 3-kinase [21], an enzyme that has previously been implicated in membrane ruffling and chemotactic responses to ligand stimulation.

The *in vivo* expression patterns of EPH subfamily receptors and their ligands would suggest that many of them function in the nervous system. Many of the receptors, including Nuk [22], have been predicted to play a role in axonogenesis. The ligand AL-1/RAGS has recently been shown to affect axonal fasciculation in one *in vitro* assay [23], and to induce growth-cone collapse and have axonal repellent activity in another [24]. Axonal guidance and targetting may prove to be one of the major functions of these receptors and their ligands. Elucidation of the functions of these receptor–ligand systems has just begun, but it seems likely that they will turn out to play important roles in the development and maintenance of many cell and tissue types.

References

1. van der Geer P, Hunter T, Lindberg RA: **Receptor protein-tyrosine kinases and their signal transduction pathways.** *Annu Rev Cell Biol* 1994, **10**:251–337.
2. Hirai H, Maru Y, Hagiwara K, Nishida J, Takaku F: **A novel putative tyrosine kinase receptor encoded by the *eph* gene.** *Science* 1987, **238**:1717–1720.
3. Winning RS, Sargent TD: **Pagliaccio, a member of the Eph family of receptor-tyrosine kinase genes, has localized expression in a subset of neural crest and neural tissues in *Xenopus laevis* embryos.** *Mech Dev* 1994, **46**:219–229.
4. Xu Q, Holder N, Patient R, Wilson SW: **Spatially regulated expression of three receptor-tyrosine kinase genes during gastrulation in the zebrafish.** *Development* 1994, **120**:287–299.
5. Tuzi NL, Gullick WJ: **Eph, the largest known family of putative growth factor receptors.** *Br J Cancer* 1994, **69**:417–421.
6. Ciossek T, Millauer B, Ullrich A: **Identification of alternatively spliced mRNAs encoding variants of MDK1, a novel receptor-tyrosine kinase expressed in the murine nervous system.** *Oncogene* 1995, **10**:97–108.
7. Valenzuela DM, Rojas E, Griffiths JA, Compton DL, Gisser M, Ip NY, *et al.*: **Identification of full-length and truncated forms of Ehk-3, a novel member of the Eph receptor-tyrosine kinase family.** *Oncogene* 1995, **10**:1573–1580.
8. Fox GM, Holst PL, Chute HT, Lindberg RA, Janssen AM, Basu R, *et al.*: **cDNA cloning and tissue distribution of five human EPH-like receptor protein-tyrosine kinases.** *Oncogene* 1995, **10**:897–905.
9. Maisonpierre PC, Barrezueta NX, Yancopoulos GD: **Ehk-1 and Ehk-2: two novel members of the Eph receptor-like tyrosine kinase family with distinctive structures and neuronal expression.** *Oncogene* 1993, **8**:3277–3288.
10. Bennett BD, Wang Z, Kuang WJ, Wang A, Groopman JE, Goeddel DV, *et al.*: **Cloning and characterization of HTK, a novel transmembrane tyrosine kinase of the EPH subfamily.** *J Biol Chem* 1994, **269**:14211–14218.
11. Andres AC, Reid HH, Zurcher G, Blaschke RJ, Albrecht D, Ziemiecki A: **Expression of two novel Eph-related receptor protein tyrosine kinases in mammary-gland development and carcinogenesis.** *Oncogene* 1994, **9**:1461–1467.
12. Bartley TD, Hunt RW, Welcher AA, Boyle WJ, Parker VP, Lindberg RA, *et al.*: **B61 is a ligand for the ECK receptor protein-tyrosine kinase.** *Nature* 1994, **368**:558–560.
13. Holzman LB, Marks RM, Dixit VM: **A novel immediate-early response gene of endothelium is induced by cytokines and encodes a secreted protein.** *Mol Cell Biol* 1990, **10**:5830–5838.
14. Kozlosky CJ, Maraskovsky E, McGrew JT, VandenBos T, Teepe M, Lyman SD, *et al.*: **Ligands for the receptor tyrosine kinases hek and elk: isolation of cDNAs encoding a family of proteins.** *Oncogene* 1995, **10**:299–306.
15. Beckmann MP, Cerretti DP, Baum P, Vanden Bos T, James L, Farrah T, *et al.*: **Molecular characterization of a family of ligands for Eph-related tyrosine kinase receptors.** *Embo J* 1994, **13**:3757–3762.
16. Davis S, Gale NW, Aldrich TH, Maisonpierre PC, Lhotak V, Pawson T, *et al.*: **Ligands for EPH-related receptor tyrosine kinases that require membrane attachment or clustering for activity.** *Science* 1994, **266**:816–819.
17. Maru Y, Hirai H, Takaku F: **Overexpression confers an oncogenic potential upon the *eph* gene.** *Oncogene* 1990, **5**:445–447.
18. Kiyokawa E, Takai S, Tanaka M, Iwase T, Suzuki M, Xiang YY, *et al.*: **Overexpression of ERK, an EPH family receptor protein-tyrosine kinase, in various human tumors.** *Cancer Res* 1994, **54**:3645–3650.
19. Lhotak V, Pawson T: **Biological and biochemical activities of a chimeric epidermal growth factor–Elk receptor tyrosine kinase.** *Mol Cell Biol* 1993, **13**:7071–7079.
20. Pandey A, Shao H, Marks RM, Polverini PJ, Dixit VM: **Role of B61, the ligand for the Eck receptor tyrosine kinase, in TNF-α-induced angiogenesis.** *Science* 1995, **268**:567–569.
21. Pandey A, Lazar DF, Saltiel AR, Dixit VM: **Activation of the Eck receptor protein tyrosine kinase stimulates phosphatidylinositol 3-kinase activity.** *J Biol Chem* 1994, **269**:30154–30157.
22. Henkemeyer M, Marengere LE, McGlade J, Olivier JP, Conlon RA, Holmyard DP, *et al.*: **Immunolocalization of the Nuk receptor tyrosine kinase suggests roles in segmental patterning of the brain and axonogenesis.** *Oncogene* 1994, **9**:1001–1014.
23. Winslow JW, Moran P, Valverde J, Shih A, Yuan JQ, Wong SC, *et al.*: **Cloning of AL-1, a ligand for an Eph-related tyrosine kinase receptor involved in axon bundle formation.** *Neuron* 1995, **14**:973–981.
24. Drechsler U, Kremoser C, Handwerker C, Löschinger J, Noda M, Bonhoeffer F: ***In vitro* guidance of retinal ganglion cell axons by RAGS, a 25 kDa tectal protein related to ligands for Eph-receptor tyrosine kinases.** *Cell* 1995, **82**:359–370.
25. Shao H, Pandey A, O'Shea KS, Seldin M, Dixit VM: **Characterization of B61, the ligand for the Eck receptor protein-tyrosine kinase.** *J Biol Chem* 1995, **270**:5636–5641.
26. Fletcher FA, Carpenter MK, Shilling H, Baum P, Ziegler SF, Gimpel S, *et al.*: **LERK-2, a binding protein for the receptor-tyrosine kinase ELK, is evolutionarily conserved and expressed in a developmentally regulated pattern.** *Oncogene* 1994, **9**:3241–3247.
27. Bennett BD, Zeigler FC, Gu Q, Fendly B, Goddard AD, Gillett N, *et al.*: **Molecular cloning of a ligand for the EPH-related receptor protein-tyrosine kinase Htk.** *Proc Natl Acad Sci USA* 1995, **92**:1866–1870.
28. Cerretti DP, Nelson N, VandenBos T, Kozlosky CJ, Reddy P, Fletcher FA: **Isolation of LERK-5: a ligand of the Eph-related receptor-tyrosine kinases.** *Mol Immunol* 1995, in press.
29. Cheng HJ, Flanagan JG: **Identification and cloning of ELF-1, a developmentally expressed ligand for the Mek4 and Sek receptor tyrosine kinases.** *Cell* 1994, **79**:157–168.
30. Shao H, Lou L, Pandey A, Verderame MF, Siever DA, Dixit VM: **cDNA cloning and characterization of a Cek7 receptor protein-tyrosine kinase ligand that is identical to the ligand (ELF-1) for the Mek-4 and Sek receptor protein-tyrosine kinases.** *J Biol Chem* 1995, **270**:3467–3470.

A. Pandey and V.M. Dixit, Department of Pathology, University of Michigan, Ann Arbor, Michigan 48109, USA. R.A. Lindberg, Department of Immunology, AMGEN, 1840 Dehavilland Drive, Thousand Oaks, California 91320, USA.